PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing: 28 October 1999 (28.10.99)	
International application No.: PCT/DK99/00209	Applicant's or agent's file reference: 21030 PC 1
International filing date: 14 April 1999 (14.04.99)	Priority date: 21 April 1998 (21.04.98)
Applicant: SØRENSEN, Kim, Ib et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

11 August 1999 (11.08.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

PLOUGMANN & VINGTOFT & PARTNERS

PATENT COOPERATION TREATY

10 AUG. 2000
HEP/LPJ
by fax and post

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

PLOUGMANN; VINGTOFT & PARTNERS
Sankt Annae Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	03.08.00
Applicant's or agent's file reference: 21030 PC 1	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK99/00209	14/04/1999	21/04/1998

Applicant: CHR. HANSEN A/S et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Büchler, S Tel.+49 89 2399-8090

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PLOUGMANN VINGTOFT & PARTNERS

10 AUG. 2000

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
21030 PC 1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK99/00209	14/04/1999	21/04/1998
International Patent Classification (IPC) or national classification and IPC C12N15/74		
Applicant CHR. HANSEN A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11/08/1999	03.08.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Buchet, A Telephone No. +49 89 2399 7401

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK99/00209

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-41 as originally filed

Claims, No.:

1-40 with telefax of 22/06/2000

Drawings, sheets:

1/10-10/10 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-40
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-40
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-40
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

D1: WO 95/10621

- D1 reports the isolation of two *Lactococcus lactis* suppressor genes: the *supD* gene is a potential serine tRNA with anticodon CTA instead of CGA, acting as an amber suppressor only recognising amber codons (p 40, l 9-27); the *supB* gene is a glutamine tRNA with anticodon ATT instead of GTT or GTC, acting as an ochre suppressor able to recognise the UAA stop codon but also the amber stop codon UAG (p 46, l 2-16). Furthermore, it was possible to isolate a purine auxotrophic *L. lactis* mutant (DN209) from strain MG1363 containing a nonsense mutation suppressed by a plasmid (pFDi17) harboring the identified ochre suppressor expressed from a functional promoter (p 49, l 6-29). The transformed strain was then able to growth in the absence of hypoxanthine in the medium (e.g. milk), revealing that this system based on the use of the suppressor gene as a marker can be applied for the long-term maintenance of plasmids during fermentation (p 50, l 8-23).
Based on the ochre suppressor gene, a food grade cloning vector named pFG1 was built, consisting of the *L. lactis* citrate plasmid replication region, the *supB* gene and a synthetic polylinker with 11 unique restriction sites (p 52, l 11-20). Its functionality was proved by cloning the *L. lactis pepN* gene leading to transformed bacteria displaying an increased lysine aminopeptidase activity (p 52, l 21-27). After successive growth on milk, a mutant plasmid conferring a faster growth to the transformed bacteria was isolated and named pFG1.1. The mutation is located in the promoter region of the *supB* gene (p 60, l 10-30), leading to its reduced expression. A mutant strain overcoming the light growth inhibition caused by pFG1 was also isolated (strain GH209).
D1 also suggests to place the expression of the suppressor gene under the control of a regulatable promoter of interest, e. g. of the *purD* gene (p 65, l 26 - p 66, l 34) or to construct a strain having an amber mutation in another essential gene (p 68, l 4-23). Isolated pure cultures of a lactic acid bacterium comprising such a vector, preferably in a concentrated form (10^9 colony per gram), and compositions containing such a culture and a carrier are intended to be used as starter cultures in the preparation of dairy

products (p 16, l 7-p 17, l 31).

1) Novelty:

- D1 discloses the *supD* gene comprising a CUA anticodon and acting as an amber suppressor but not a derivative food grade vector. Therefore, claims 1 to 40 meet the requirements of Article 33.2 PCT.

2) Inventive step:

- The technical problem to be solved by the present invention as well as by D1, considered as the closest prior art, is to provide an alternative cloning system for stably maintaining a cloning plasmid in any lactic acid bacterium.

- The solution disclosed in the present invention is the use of a food grade vector harboring an amber suppressor gene (e. g. *supD*) in strains having a compatible non-sense mutation in an essential gene (e. g. *pyrF*).

- It was not inventive per se to replace in the food grade vector disclosed in D1 the ochre suppressor gene by the alternative suppressor gene cloned in D1 (*supD*). However, besides the fact that the amber suppressor gene selectively suppresses amber mutations, it is surprising that the resulting vectors can have the following advantageous properties: they are stable and they can have at least one of the properties, relative to their growth or metabolic capacities, cited in claim 1 (i to iii). These features (parameters) have to be incorporated in claim 1 since all the vectors having only the structural feature ("amber suppressor ... CUA anticodon") are not necessary advantageous: D1 e.g. shows that the growth rate of a strain transformed by such a vector can depend on the background of the strain itself or on the promoter of the suppressor gene.

- Therefore, claims 1 to 40 are considered to fulfil the requirements of Article 33.3 PCT.

21030PC1

Chr. Hansen A/S
International Patent Application No. PCT/DK99/00209
Publication No. WO 99/54488

NEW CLAIMS, June 2000

1. A recombinant vector consisting essentially of lactic acid bacterial DNA, the vector comprising a gene coding for an amber suppressor which is a tRNA comprising the CUA anticodon and a replicon making the vector capable of replicating in a lactic acid bacterium, the vector having at least one of the following characteristics:

(i) when it is present in *Lactococcus lactis* strain FA4-1-1 (DSM 12086) having an amber mutation in the *pyrF* gene that is suppressible by the suppressor, it permits said strain to grow at 30°C at a doubling time of at the most 100 minutes in a minimal medium not containing pyrimidine sources;

(ii) when it is present in a strain of *Lactococcus lactis* FH CY-1 that has an amber mutation in the *pyrF* gene (strain CHCC4146, DSM 12109), the amber mutation being suppressible by the suppressor, it permits the strain to acidify milk under identical conditions at essentially the same rate of that of the parent strain (FH CY-1, DSM 12087);

(iii) it permits the *Lactococcus lactis* FA4-1-1 strain to grow at 30°C in a minimal medium not containing pyrimidine sources at a doubling time which is less than that for the *Lactococcus lactis* strain DN209 transformed with the vector pFG1.1 (DSM 12088), the pFG1.1 vector comprising a gene coding for a suppressor that is capable of suppressing the amber mutation in the DN209 strain, the transformed DN209 strain growing under conditions identical to those for the FA4-1-1 strain.

2. A vector according to claim 1 which has at least two of the characteristics (i) to (iii).

3. A vector according to claim 1 which has the characteristics (i) to (iii).

4. A vector according to claim 1 wherein the gene coding for the suppressor is derived from the chromosome of a lactic acid bacterium.

5. A vector according to claim 4 wherein the gene coding for a suppressor is under the control of a regulatable promoter.

6. A vector according to claim 5 wherein the regulatable promoter is a promoter not naturally related to the gene.

7. A vector according to claim 1 wherein the amber suppressor results from at least one change of nucleotide in an anticodon.

8. A vector according to claim 7 wherein the suppressor has two or three changes of nucleotide.

9. A vector according to claim 1 wherein the suppressor is a suppressor selected from the group consisting of a *supD*, *supE*, *supF*, *supP*, *supU* and a *supZ* suppressor.

10. A vector according to claim 1 wherein the replicon is derived from a *Lactococcus lactis* plasmid.

11. A vector according to claim 1 that comprises at least one unique restriction site.

12. A vector according to claim 1 that comprises a multiple cloning site.

13. A vector according to claim 1 which is a theta-replicating plasmid.

14. A vector according to claim 1 which is stably maintained for at least 35 generations in a lactic acid bacterium cultivated in a medium not containing pyrimidine sources.

15. A vector according to any of claims 1-14 which is selected from the group consisting of pFG100 deposited under the accession No. DSM 12091, a mutant, variant or derivative of pFG100, pFG200 deposited under the accession No. DSM 12108 and a mutant, variant or derivative of pFG200, said mutants, variants or derivatives essentially having the characteristics of the respective vector from which they are derived.

16. A vector according to claim 1 which comprises a gene coding for a desired gene product.

17. A vector according to claim 16 wherein the gene product is a peptidase selected from the group consisting of lysine-aminopeptidase, glutamyl-aminopeptidase, cysteine-aminopeptidase, iminopeptidase, X-prolyl-dipeptidyl aminopeptidase, endopeptidase, dipeptidase and tripeptidase.

18. A vector according to claim 16 wherein the gene product confers bacteriophage resistance to a lactic acid bacterial host cell.

19. A vector according to claim 16 wherein the gene product is a bacteriophage lysin.

20. A vector according to claim 19 wherein the gene coding for the bacteriophage lysin is derived from the bacteriophage ØvML3 as contained in DN209/pFG7 deposited under the accession No. DSM 12089.

21. A vector according to claim 16 wherein the gene product is involved in nisin synthesis or nisin resistance.

22. A lactic acid bacterium comprising a vector according to any of claims 1-21.

23. A lactic acid bacterium according to claim 22 that comprises an amber mutation being suppressible by the nonsense amber suppressor.

24. A lactic acid bacterium according to claim 23 wherein the amber mutation is located on a replicon different from the one containing the gene coding for the nonsense suppressor.

25. A lactic acid bacterium according to claim 22 wherein the suppressor is one suppressing a nonsense mutation which in the absence of a nonsense suppressor capable of suppressing the mutation, confers auxotrophy.

26. A lactic acid bacterium according to claim 25 wherein the nonsense mutation is in a gene involved in the synthesis of pyrimidine nucleotides.

27. A lactic acid bacterium according to claim 26 wherein the nonsense mutation is in a *pyr* gene.

28. A lactic acid bacterium according to claim 22 which is selected from the group consisting of a *Lactococcus* sp., *Streptococcus* sp., *Lactobacillus* sp., *Leuconostoc* sp., *Pedlococcus* sp. and *Bifidobacterium* sp.

29. A lactic acid bacterium according to claim 28 which is *Lactococcus lactis*.

30. A lactic acid bacterium according to claim 29 which is *Lactococcus lactis* subsp. *lactis* strain FA4-1-1 containing pFG100, deposited under the accession No. DSM 12091 or *Lactococcus lactis* subsp. *lactis* strain CHCC4146 containing pFG200, deposited under the accession No. DSM 12108.

31. A lactic acid bacterium according to claim 22 wherein the vector is stably maintained for at least 35 generations when it is cultivated in a medium not containing pyrimidine sources.

32. An isolated pure culture of a lactic acid bacterium according to any of claims 22-31.

33. A composition comprising an isolated pure culture of a lactic acid bacterium as defined in claim 32, and a carrier.

34. A composition according to claim 33 containing at least 10^5 colony forming units of the lactic acid bacterium per g.

35. A method of using a composition as defined in claim 33 as a starter culture in the preparation of a product selected from the group consisting of a dairy flavour, a product for cheese flavouring, a food product and a feed product.

36. A method of stably maintaining a vector according to claim 1 in lactic acid bacterial host cells growing in a particular environment, comprising providing said host cells as nonsense mutant cells having lost the capability of growing in said environment, and transformed with the vector according to claim 1 containing a nonsense suppressor gene encoding a gene product restoring the capability of the nonsense mutant cells to grow in said environment whereby, if the vector is lost from the lactic acid bacterial cells, the cells will not grow.

37. A method according to claim 36 wherein the nonsense mutant cells having lost the capability to grow are auxothrophic cells.

38. A method according to claim 37 wherein the nonsense mutant cells have a mutation in a gene involved in the synthesis of nucleotides.

39. A method according to claim 38 wherein the lactic acid bacterial host cells are *pyr* mutants.

40. A method according to claim 36 wherein the environment is a material selected from the group consisting of milk, a vegetable material, a meat product, a must, a fruit juice, a wine, a dough and a batter.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
21030 PC 1		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/DK 99/ 00209	14/04/1999	21/04/1998
Applicant		
CHR. HANSEN A/S et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

FOOD-GRADE CLONING VECTOR AND THEIR USE IN LACTIC ACID BACTERIA

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 5

[X] as suggested by the applicant.

[ ] None of the figures.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/DK 99/00209

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/74 C12N15/68 C12N9/88 C12N9/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 10621 A (CHR HANSEN S LAB DANMARK ;DICKELY FRANCOISE (FR); JOHANSEN ERIC (D) 20 April 1995 (1995-04-20) cited in the application	1-15, 17-19, 23-30, 32-41
Y	the whole document ---	20-22
Y	WO 90 00599 A (AGRICULTURAL & FOOD RES) 25 January 1990 (1990-01-25) cited in the application the whole document ---	20,21
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 July 1999	22/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Smalt, R

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	KUIPERS O P ET AL: "CHARACTERIZATION OF THE NISIN GENE CLUSTER NISABTCIPR OF LACTOCOCCUS LACTIS REQUIREMENT OF EXPRESSION OF THE NISA AND NISI FOR DEVELOPMENT OF IMMUNITY" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 216, no. 1, 1 August 1993 (1993-08-01), pages 281-291, XP002041580 ISSN: 0014-2956 abstract; figure 2 ---	22
Y	WO 91 09131 A (VALIO FINNISH COOPERATIVE DAIR ;WESSELS STEPHEN (DK); JOSEPHSEN JY) 27 June 1991 (1991-06-27) abstract ---	22
X	DICKELY F ET AL: "ISOLATION OF LACTOCOCCUS LACTIS NONSENSE SUPPRESSORS AND CONSTRUCTION OF A FOOD-GRADE CLONING VECTOR" MOLECULAR MICROBIOLOGY, vol. 15, 1 January 1995 (1995-01-01), pages 839-847, XP000572225 cited in the application	1-5, 7-15,17, 18, 23-27, 29,30, 32-39,41
Y	the whole document ---	6
Y	WO 94 16086 A (BIOTEKNOLOGISK INST ;HANSENS LAB (DK); ISRAELSEN HANS (DK); HANSEN) 21 July 1994 (1994-07-21) the whole document ---	6
X	JOHANSEN, E. ET AL.: "Nonsense suppression in Lactococcus lactis: construction of a <<food grade>> cloning vector." DEVELOPMENTS IN BIOLOGICAL STANDARDIZATION, vol. 85, 1995, pages 531-4, XP002085666 cited in the application the whole document ---	1-4, 7-15,17, 18, 23-26, 29,30, 32-39,41
A	ANDERSEN, P.S. ET AL.: "Sequence analysis and identification of the pyrKDbF operon from Lactococcus lactis include a novel gene, pyrK, involved in pyrimidine biosysnthesis." JOURNAL OF BACTERIOLOGY, vol. 178, no. 16, August 1996 (1996-08), pages 5005-12, XP002085667 cited in the application the whole document -----	28,29, 39,40

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
[redacted]/DK 99/00209

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9510621 A	20-04-1995	US 5691185 A	25-11-1997
		AU 684556 B	18-12-1997
		AU 7852994 A	04-05-1995
		CA 2160166 A	20-04-1995
		EP 0722503 A	24-07-1996
		NZ 274481 A	20-12-1997
		US 5866385 A	02-02-1999
WO 9000599 A	25-01-1990	AT 130031 T	15-11-1995
		AU 623166 B	07-05-1992
		AU 4037789 A	05-02-1990
		DE 68924775 D	14-12-1995
		DK 5491 A	11-01-1991
		EP 0425572 A	08-05-1991
		GB 2243611 A,B	06-11-1991
		IE 71020 B	15-01-1997
		US 5360617 A	01-11-1994
WO 9109131 A	27-06-1991	AT 102652 T	15-03-1994
		AU 645459 B	13-01-1994
		AU 7045991 A	18-07-1991
		CA 2072007 A	21-06-1991
		DE 69007298 D	14-04-1994
		DE 69007298 T	30-06-1994
		WO 9109132 A	27-06-1991
		DK 506789 T	06-06-1994
		EP 0506789 A	07-10-1992
		ES 2062757 T	16-12-1994
		IE 66032 B	29-11-1995
		US 5580787 A	03-12-1996
WO 9416086 A	21-07-1994	AU 675821 B	20-02-1997
		AU 5832594 A	15-08-1994
		CA 2152898 A	21-07-1994
		EP 0677110 A	18-10-1995
		JP 8500739 T	30-01-1996
		NZ 259510 A	24-06-1997
		NZ 286635 A	24-06-1997
		US 5837509 A	17-11-1998

CLAIMS

1. A recombinant vector consisting essentially of lactic acid bacterial DNA, the vector comprising a gene coding for a tRNA comprising an amber suppressor and a replicon making the vector capable of replicating in a lactic acid bacterium, the vector having at least one of the following characteristics:

(i) when it is present in *Lactococcus lactis* strain FA4-1-1 (DSM 12086) having an amber mutation in the *pyrF* gene that is suppressible by the suppressor, it permits said strain to grow at 30°C at a doubling time of at the most 100 minutes in a minimal medium not containing pyrimidine sources;

(ii) when it is present in a strain of *Lactococcus lactis* FH CY-1 that has an amber mutation in the *pyrF* gene (strain CHCC4146, DSM 12109), the amber mutation being suppressible by the suppressor, it permits the strain to acidify milk under identical conditions at essentially the same rate of that of the parent strain (FH CY-1, DSM 12087);

(iii) it permits the *Lactococcus lactis* FA4-1-1 strain to grow at 30°C in a minimal medium not containing pyrimidine sources at a doubling time which is less than that for the *Lactococcus lactis* strain DN209 transformed with the vector pFG1.1 (DSM 12088), the pFG1.1 vector comprising a gene coding for a suppressor that is capable of suppressing the amber mutation in the DN209 strain, the transformed DN209 strain growing under conditions identical to those for the FA4-1-1 strain.

2. A vector according to claim 1 which has at least two of the characteristics (i) to (iii).

3. A vector according to claim 1 which has the characteristics (i) to (iii).

4. A vector according to claim 1 wherein the gene coding for the nonsense suppressor is derived from the chromosome of a lactic acid bacterium.

5. A vector according to claim 4 wherein the gene coding for a nonsense suppressor is under the control of a regulatable promoter.

6. A vector according to claim 5 wherein the regulatable promoter is a promoter not naturally related to the gene.

7. A vector according to claim 1 wherein the amber suppressor results from at least one change of nucleotide in an anticodon.

8. A vector according to claim 7 wherein the suppressor has two or three changes of nucleotide.

9. A vector according to claim 7 or 8 wherein the suppressor comprises a CUA anticodon.

10. A vector according to claim 9 wherein the suppressor is a suppressor selected from the group consisting of a *supD*, *supE*, *supF*, *supP*, *supU* and a *supZ* suppressor.

11. A vector according to claim 1 wherein the replicon is derived from a *Lactococcus lactis* plasmid.

12. A vector according to claim 1 that comprises at least one unique restriction site.

13. A vector according to claim 1 that comprises a multiple cloning site.

14. A vector according to claim 1 which is a theta-replicating plasmid.

15. A vector according to claim 1 which is stably maintained for at least 35 generations in a lactic acid bacterium cultivated in a medium not containing pyrimidine sources.

16. A vector according to any of claims 1-15 which is selected from the group consisting of pFG100 deposited under the accession No. DSM 12091, a mutant, variant or derivative of pFG100, pFG200 deposited under the accession No. DSM 12108 and a mutant, variant or derivative of pFG200, said mutants, variants or derivatives essentially having the characteristics of the respective vector from which they are derived.

17. A vector according to claim 1 which comprises a gene coding for a desired gene product.

18. A vector according to claim 17 wherein the gene product is a peptidase selected from the group consisting of lysine-aminopeptidase, glutamyl-aminopeptidase, cysteine-

aminopeptidase, iminopeptidase, X-prolyl-dipeptidyl aminopeptidase, endopeptidase, dipeptidase and tripeptidase.

19. A vector according to claim 17 wherein the gene product confers bacteriophage resistance to a lactic acid bacterial host cell.

20. A vector according to claim 17 wherein the gene product is a bacteriophage lysin.

21. A vector according to claim 20 wherein the gene coding for the bacteriophage lysin is derived from the bacteriophage ØvML3 as contained in DN209/pFG7 deposited under the accession No. DSM 12089.

22. A vector according to claim 17 wherein the gene product is involved in nisin synthesis or nisin resistance.

23. A lactic acid bacterium comprising a vector according to any of claims 1-22.

24. A lactic acid bacterium according to claim 23 that comprises an amber mutation being suppressible by the nonsense amber suppressor.

25. A lactic acid bacterium according to claim 24 wherein the amber mutation is located on a replicon different from the one containing the gene coding for the nonsense suppressor.

26. A lactic acid bacterium according to claim 23 wherein the suppressor is one suppressing a nonsense mutation which in the absence of a nonsense suppressor capable of suppressing the mutation, confers auxotrophy.

27. A lactic acid bacterium according to claim 26 wherein the nonsense mutation is in a gene involved in the synthesis of pyrimidine nucleotides.

28. A lactic acid bacterium according to claim 27 wherein the nonsense mutation is in a *pyr* gene.

29. A lactic acid bacterium according to claim 23 which is selected from the group consisting of a *Lactococcus* sp., *Streptococcus* sp., *Lactobacillus* sp., *Leuconostoc* sp., *Pediococcus* sp. and *Bifidobacterium* sp.

30. A lactic acid bacterium according to claim 29 which is *Lactococcus lactis*.

31. A lactic acid bacterium according to claim 30 which is *Lactococcus lactis* subsp. *lactis* strain FA4-1-1 containing pFG100, deposited under the accession No. DSM 12091 or *Lactococcus lactis* subsp. *lactis* strain CHCC4146 containing pFG200, deposited under the accession No. DSM 12108.

32. A lactic acid bacterium according to claim 23 wherein the vector is stably maintained for at least 35 generations when it is cultivated in a medium not containing pyrimidine sources.

33. An isolated pure culture of a lactic acid bacterium according to any of claims 23-32.

34. A composition comprising an isolated pure culture of a lactic acid bacterium as defined in claim 33, and a carrier.

35. A composition according to claim 34 containing at least 10^5 colony forming units of the lactic acid bacterium per g.

36. A method of using a composition as defined in claim 34 as a starter culture in the preparation of a product selected from the group consisting of a dairy flavour, a product for cheese flavouring, a food product and a feed product.

37. A method of stably maintaining a vector according to claim 1 in lactic acid bacterial host cells growing in a particular environment, comprising providing said host cells as nonsense mutant cells having lost the capability of growing in said environment, and transformed with the vector according to claim 1 containing a nonsense suppressor gene encoding a gene product restoring the capability of the nonsense mutant cells to grow in said environment whereby, if the vector is lost from the lactic acid bacterial cells, the cells will not grow.

38. A method according to claim 37 wherein the nonsense mutant cells having lost the capability to grow are auxothrophic cells.

39. A method according to claim 38 wherein the nonsense mutant cells have a mutation in a gene involved in the synthesis of nucleotides.

40. A method according to claim 39 wherein the lactic acid bacterial host cells are *pyr* mutants.

41. A method according to claim 37 wherein the environment is a material selected from the group consisting of milk, a vegetable material, a meat product, a must, a fruit juice, a wine, a dough and a batter.